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# Novel phthalocyanine-based polymeric micelle with high near-infrared photothermal conversion efficiency under 808 nm laser irradiation for in vivo cancer therapy

Lu Li,[‡a] Qingzhu Yang,[‡b] Lei Shi,[a] Nannan Zheng,[b] Zeyu Li,[a] Kai Li,[b] Shupei Qiao,[b] Tao Jia,[*a] Tiedong Sun[*a] and Yue Wang[c]

a. Department of Chemistry, College of Science, Northeast Forestry University, 26 Hexing Road, Harbin 150040, P. R. China. E-mail: jiataopolychem@nefu.edu.cn; tiedongsun@nefu.edu.cn

b. School of Life Science and Technology, Harbin Institute of Technology, Harbin 150080, China

c. State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Qianjin Street, Changchun, P. R. China.

**General materials characterization:**

Materials and methods: $^{1}H$ NMR spectra were measured on the AVANCEIIIHD 500 MHz spectrometer (USA) with tetramthylsilane as the internal standard. $^{13}C$ NMR spectra were measured on the AVANCEIIIHD 125 MHz spectrometer (USA) with tetramthylsilane as the internal standard. MALDI-TOF mass spectra were recorded on the Kratos AXIMA-CFR KompactMALDI mass spectrometer with anthracene-1,8,9-triol as the matrix. The optical characteristics of 4OCSPC and 4OCSPC/F127 were investigated by UV−vis absorption spectra (TU1901 UV−vis spectrophotometer, Beijing PuXi general instrument co., LTD, China). The size distribution of resulting micelles was determined by dynamic light scattering (DLS) using a NanoBrook 90 Plus zeta (Brookhaven, USA) with angle detection at 90°. The TEM images were collected on a field emission high-resolution JEM-2100 transmission electron microscope (JEOL, Japan) operating at an acceleration voltage of 200 kV. Photothermal temperature was recorded by an IR thermal camera (E50, IRS Systems). All reagents and solvents, unless otherwise specified, were obtained from Aldrich (USA) and Acros (Belgium), and were used as received. All reactions were carried out using Schlenk techniques under a nitrogen atmosphere. 4OCSPC was synthesized as plotted in Scheme 1, where Cl-4OCPN was prepared according to the method reported.[1] Details of synthesis and characterization are presented as follows.

**Synthesis of 4OCSPC:**

Cl-4OCPN (3.40 g, 10.0 mmol) and tributyl (4-hexylthiophen-2-yl) stannane (7.64 g,

16.6 mmol) and tetrakis (triphenylphosphine) palladium (0) (1.14 g, 1.0 mmol) were dissolved in degassed 100 mL toluene, and the mixture was stirred at 80$^{o}$C for 8 h. The reaction mixture was allowed to cool to room temperature, solvent was removed by vacuum evaporation and the crude product was purified by column chromatography using silica gel with dichloromethane and petroleum ether as the eluents to obtain 4OCSPN (4.35 g, Yield: 72.0%). Then 4OCSPN (3.02 g, 5.0 mmol) was dissolved in 15 mL dry n-pentanol and the mixture was heated at 100$^{o}$C for 5 min. Under nitrogen atmosphere, lithium (0.11 g, 15.0 mmol) was added, and then the mixture was refluxed for 2 h. After cooling to room temperature, solvent was removed by vacuum evaporation and the crude product was purified by column chromatography using silica gel with dichloromethane and petroleum ether as the eluents to obtain the dark green powder. Yield: 293 mg (10%). $^{1}$H NMR (500 MHz, $CDCl_3$, δ): 7.07 (s, 8 H), 7.02 (s, 8 H), 4.74 (m, 16 H), 1.85 (m, 16 H), 1.64 (m, 16 H), 1.36 – 1.29 (m, 80 H), 0.94 (m, 24 H), 0.88 (m, 24 H). MS (MALDI-TOF): m/z: 2460.3 $[M + K]^{+}$.

**Synthesis of the polymeric micelle 4OCSPC/F127:**

A mixture of 2 mg of 4OCSPC and 40 mg Pluronic 127 was completely dissolved in 1 mL of THF for 2 h. Then 10 mL of deionized-water was quickly injected into the mixture under vigorous stirring at room temperature. After being stirred for 5 min, the dispersion was dialyzed against deionized-water by 3.5 KDa dialysis membranes for 48 h to remove THF. The 4OCSPC/F127 aqueous solutions were separated by

centrifugation at 10000 rpm for 5 min to remove unencapsulated surfactant and then redispersed in deionized-water before characterization and cell study.

**Synthesis of the polymeric micelle 4OCSPC + DiR/F127:**

A mixture of 1 mg of 4OCSPC, 1 mg DiR and 20 mg Pluronic 127 was completely dissolved in 0.5 mL of THF and 0.5 mL DMSO for 2 h. Then 5 mL of deionized-water was quickly injected into the mixture under vigorous stirring at room temperature. After being stirred for 5 min, the dispersion was dialyzed against deionized-water by 3.5 KDa dialysis membranes for 48 h to remove solvent. The 4OCSPC + DiR/F127 aqueous solutions were separated by centrifugation at 10000 rpm for 5 min to remove unencapsulated surfactant and then redispersed in deionized-water before characterization and cell study.

**Photothermal evaluation:**

The stock dispersion of 4OCSPC/F127 was diluted to 1.0, 2.0, 5.0 and 10.0 μg/mL, respectively. At each concentration, a total of 1 mL dispersion was used in the evaluation of its photothermal effect under irradiation of a 808-nm laser with a power density of 0.7 W/cm$^2$. Every sample was irradiated for 10 min and allowed to cool down to the room temperature for the next 20 min, which was counted as one cycle, PBS served as the control group. The photothermal conversion efficiency of 4OCSPC was calculated with the following equation:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_s}{I(I - 10^{-A_{808}})}$$

where h is the heat transfer coefficient, S is the surface area of the container, $T_{max}$ is the maximum temperature of the 4OCSPC/F127, and $T_{surr}$ is the temperature of the surrounding. I is the laser power, $A_\lambda$ is the absorbance of 4OCSPC at 808 nm, $Q_s$ is the heat change of the solvent, and η is the photothermal conversion efficiency.

**Cell labeling**

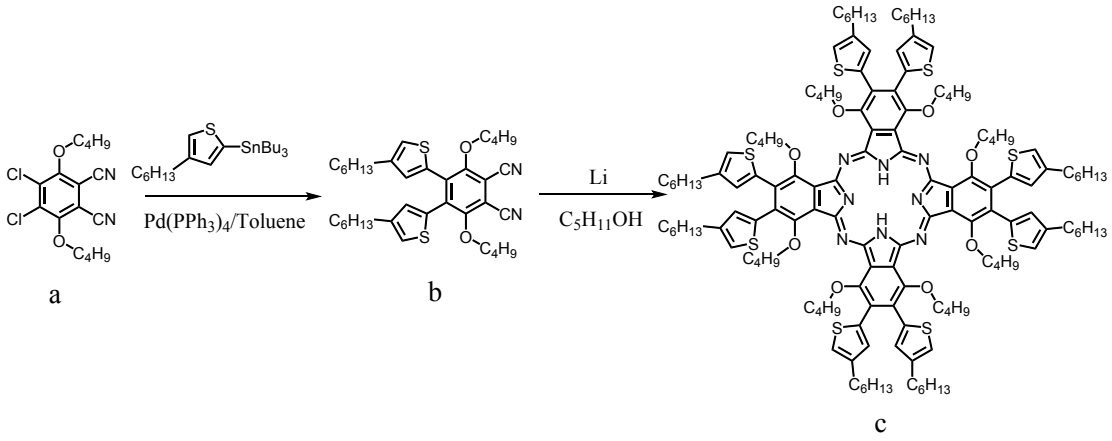
HeLa cells were seeded in a 6-well plate, preincubated for 12 h. Then incubated with 4OCSPC/F127 for 6 h. The cells were treated with 808 nm laser irradiation for 5 min and washed with PBS three times. Next the cells were stained with PI and calcein AM for 15 min and washed with PBS three times. The fluorescence microscopy (Olympus, BX51) was used to observe the state of cells. Cells with PBS were used as control.

**Cytotoxicity Evaluation in Vitro:**

HeLa cells were seeded in a 96-well plate, preincubated for 12 h. And then incubated with 4OCSPC/F127 for 24 h at concentrations ranging from 0 to 40.0 μg/mL. The cells were treated with 808 nm laser irradiation for 5 min. The DMEM medium was replaced with 20 μl 0.5 mg/ml MTT and after 4 h the MTT solution was replaced with 150 μl DMSO solution. Cell viability was measured at 490 nm by colorimetric assay (Rayto RT-2100C, Shenzhen, China). Cells without irradiation in medium were used as control.

***In Vivo* Photothermal Therapy:**

Female BALB/c mice (6-8 week old, 18 to 20 g body weight) were purchased from the 2nd Affiliated Hospital of Harbin Medical University Center (Harbin, China), maintained under pathogen-free conditions and allowed free access to sterile food and water. All *in vivo* studies were performed in accordance with the ‘‘Rules for experiments animals’’ published by the Chinese Government (Beijing, China). The tumor model was established by injecting $1 \times 10^6$ 4T1 cells into the right flank of each mouse. Tumor nodules were allowed to grow to a volume of ~100 $mm^3$ before treatments. Tumor-bearing BALB/c mice were randomly assigned to 4 groups, and each group had five mice. Tumor length and width were measured with calipers, and the tumor volume was calculated using the equation $V = (a \times b^2)/2$, in which a was the length and b was the width. Test animals received one intratumoral injection of PBS without or with 808 nm laser irradiation, 4OCSPC/F127 in PBS (40 μg/mL) without or with 808 nm laser irradiation on first day. The tumor volume of each mouse were measured every two days.




**Scheme 1.** The synthetic route of 4OCSPC.

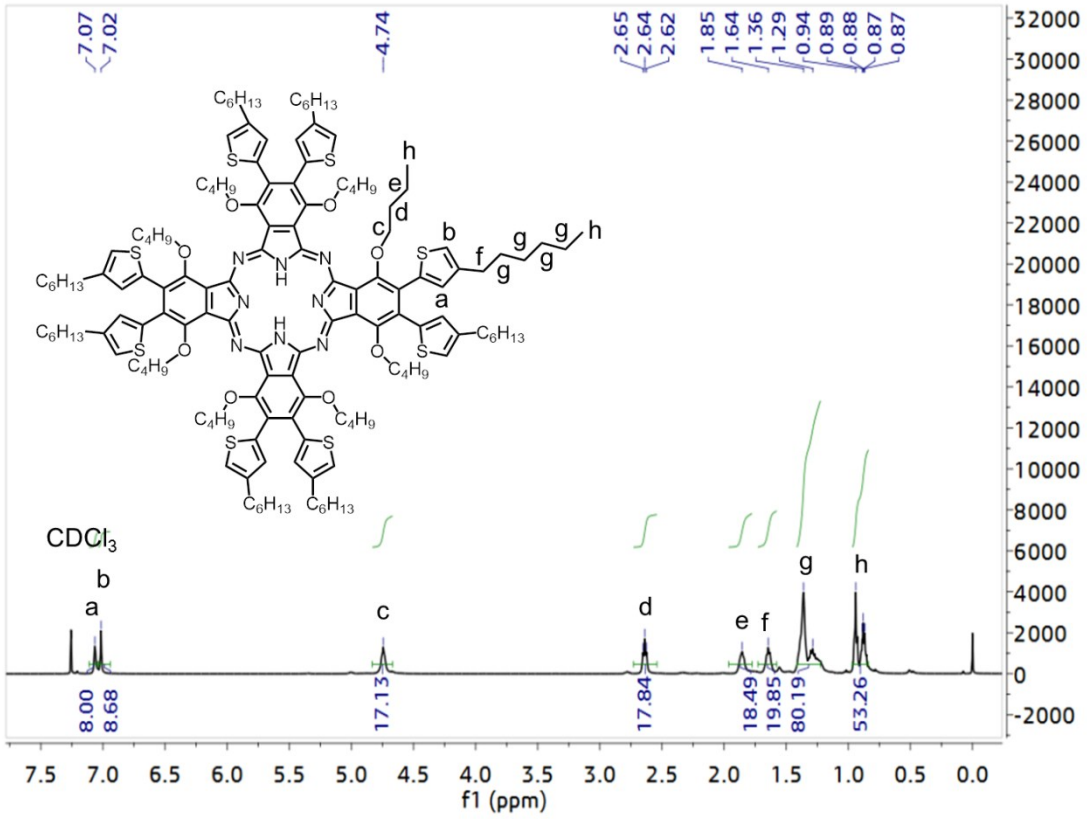



**Figure S1.** $^{1}$H NMR spectrum of 4OCSPC in $CDCl_3$.

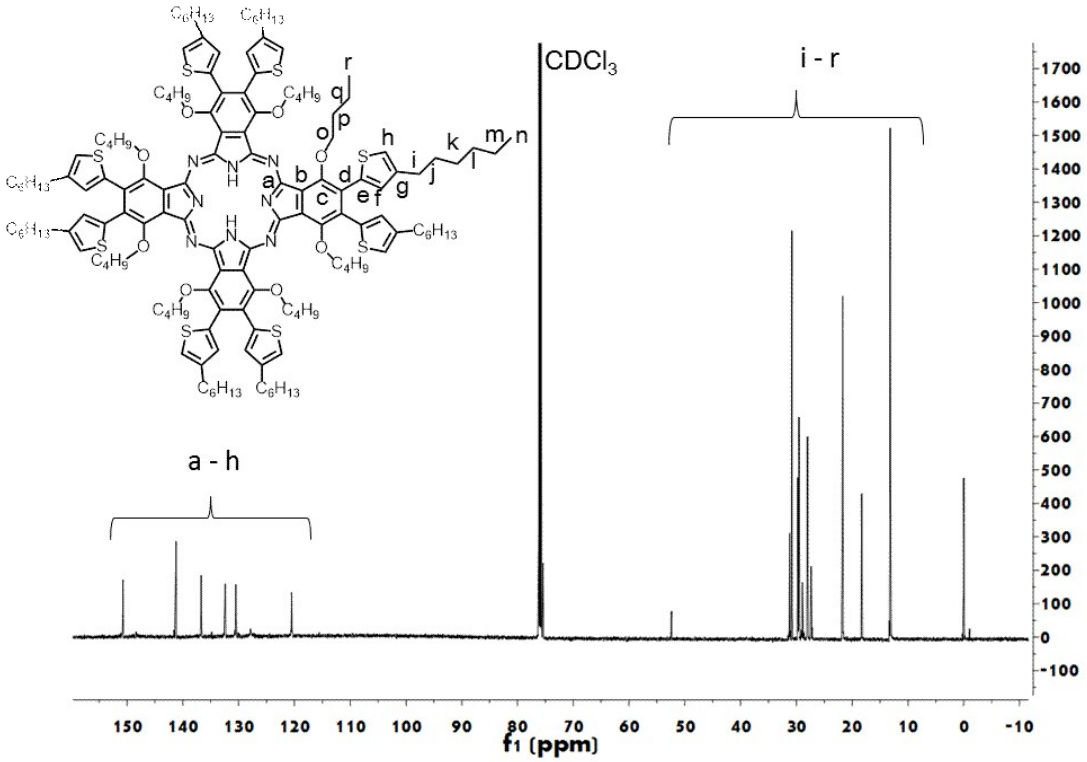



**Figure S2.** $^{13}C$ NMR spectrum of 4OCSPC in $CDCl_3$.

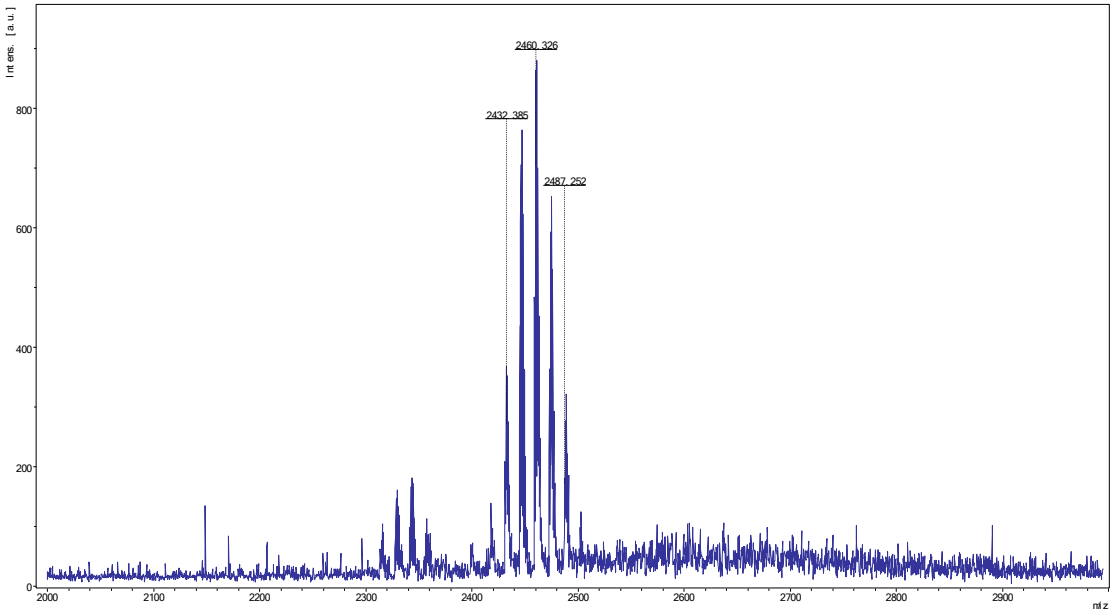



**Figure S3.** MADLI-TOF mass spectrum of 4OCSPC.

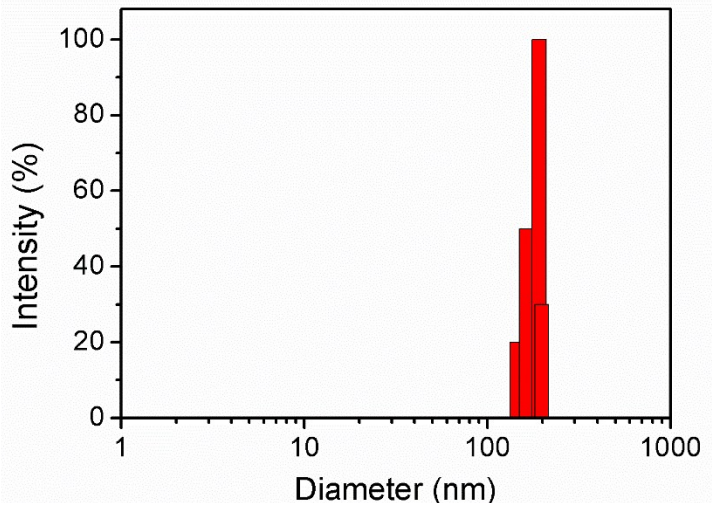



**Figure S4.** The dynamic light scattering (DLS) spectrum of 4OCSPC/F127 micelle ($C_{4OCSPC}$ = 20.0 μg/mL)

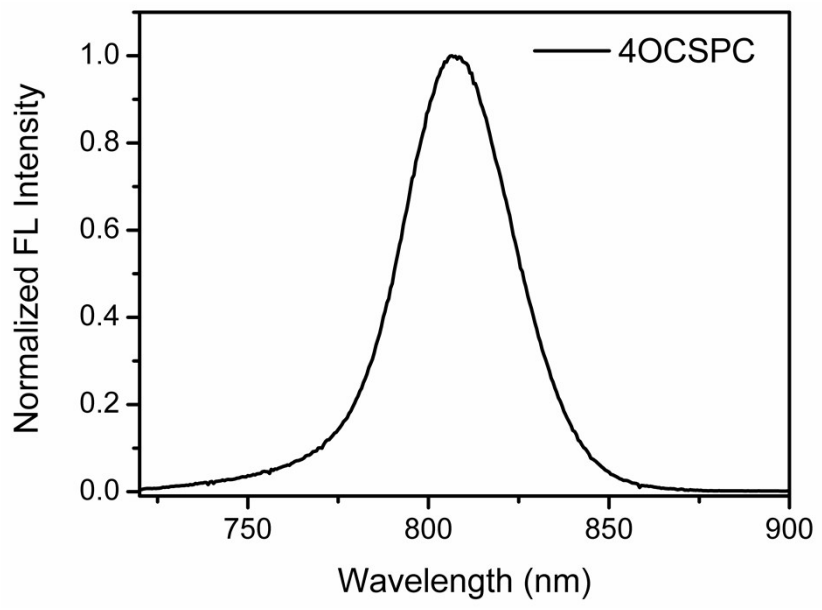



**Figure S5.** The fluorescence emission spectrum of 4OCSPC in THF (C = 20 μg/mL).

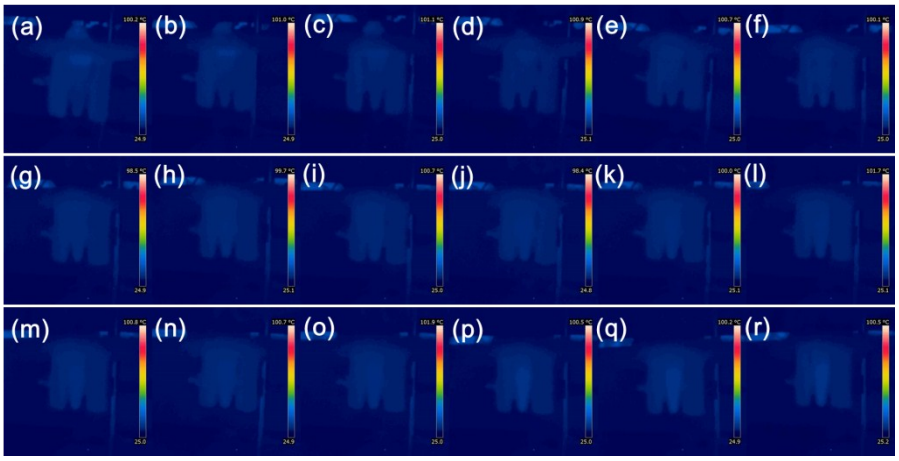



**Figure S6.** Thermal imaging of PBS under 808 nm laser irradiation (0.7 W/cm$^2$) for different times, a: 0 s; b: 5 s; c: 10 s; d: 15 s; e: 20 s; f: 25 s; g: 30 s; h: 40 s; i: 60 s; j: 120 s; k: 180 s; l: 240 s; m: 300 s; n: 360 s; o: 420 s; p: 480 s; q: 540 s; r: 600 s.

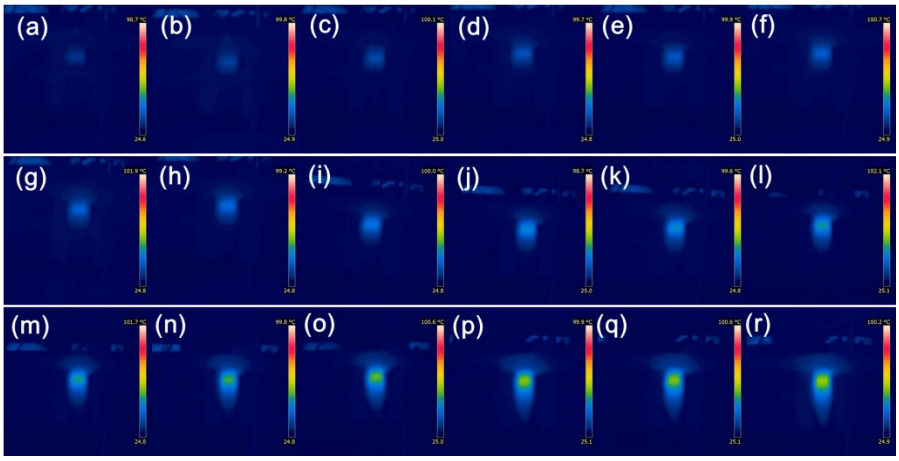



**Figure S7.** Thermal imaging of 4OCSPC/F127 micelle ($C_{4OCSPC}$ = 5 μg/mL) under 808 nm laser irradiation (0.7 W/cm$^2$) for different times, a: 0 s; b: 5 s; c: 10 s; d: 15 s; e: 20 s; f: 25 s; g: 30 s; h: 40 s; i: 60 s; j: 120 s; k: 180 s; l: 240 s; m: 300 s; n: 360 s; o: 420 s; p: 480 s; q: 540 s; r: 600 s.

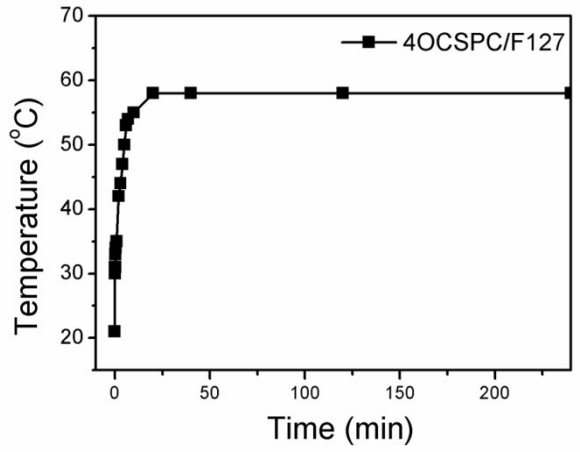



**Figure S8.** The photothermal heating curve of 4OCSPC/F127 solutions ($C_{4OCSPC}$ = 5.0 μg/mL) under 808 nm continuous laser irradiation at the power density of 0.7 W/cm$^2$ for 4 h.

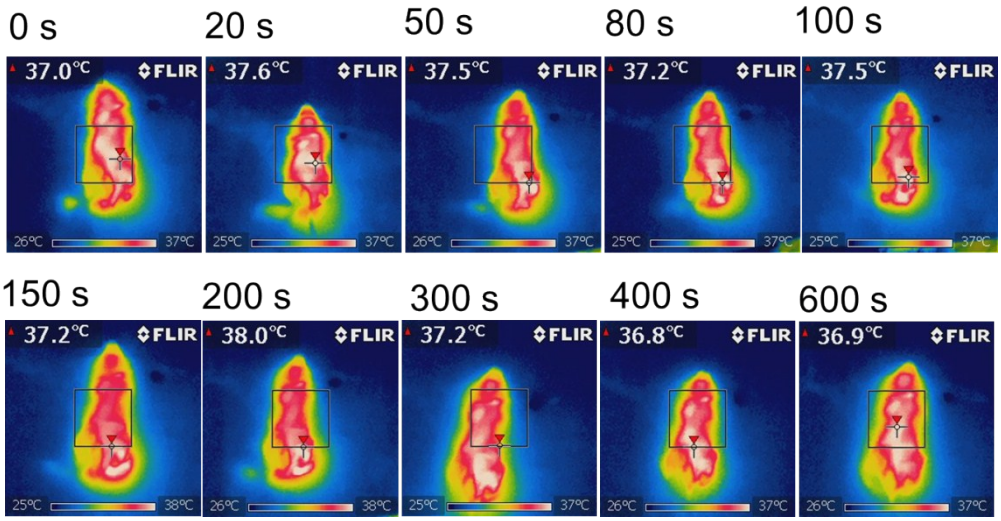



**Figure S9.** Thermal imaging of mice without any treatments under 808 nm laser irradiation for different times.

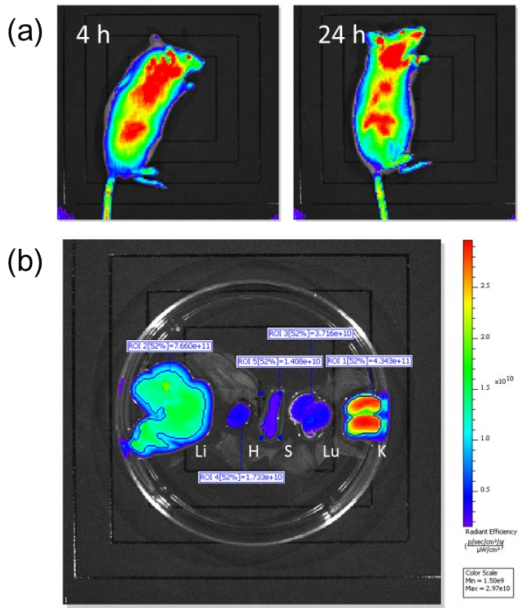
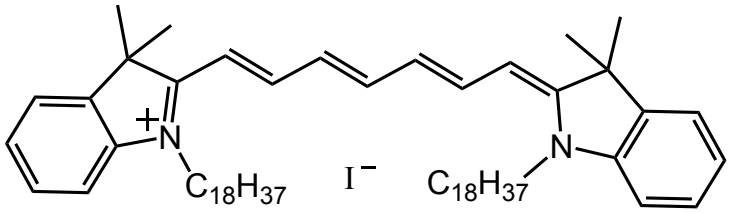



Figure S10 (a) *In vivo* images of mice 4 h and 24 h after treatment (intravenous injection) with DiR-loaded micelles (4OCSPC + DiR/F127; 200 ng/mL, 200 μL). (b) *Ex vivo* images of tissues 24 h after DiR treatment (Li, liver; H, heart ; S, spleen; Lu, lung; K, kidney).

Notes:

The chemical structure of DiR:



## References:

1. J. P. Fox and D. P. Goldberg, *Inorg. Chem.*, 2003, **42**, 8181.